Improvement in In Vitro Insulin Action After One Month of Insulin Therapy in Obese Noninsulin-dependent Diabetics

MEASUREMENTS OF GLUCOSE TRANSPORT AND METABOLISM, INSULIN BINDING, AND LIPOLYSIS IN ISOLATED ADIPOCYTES

J. E. FOLEY, A. KASHIWAGI, M. A. VERSO, G. REAVEN, and J. ANDREWS, *Phoenix Clinical Research Section, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona 85016*

ABSTRACT It has been previously reported that maximum insulin-stimulated glucose transport and utilization were both decreased, while basal lipolysis was increased in adipocytes from obese subjects with noninsulin-dependent diabetes mellitus (NIDDM). To determine whether these values can be returned towards those obtained in equally obese subjects with normal glucose tolerance, these measures of adipocyte metabolism were quantified in 10 NIDDM subjects before and after control of hyperglycemia with insulin. The results demonstrate that maximum insulin-stimulated glucose transport ($P < 0.02$) and glucose incorporation into triglyceride ($P < 0.01$) and CO_2 ($P < 0.05$) (at 5.5 mM glucose) increased and basal lipolysis decreased ($P < 0.05$) after 4 wk of insulin treatment. In contrast, glucose incorporation into lactate and other glycolytic metabolites (at 5.5 mM glucose), and sensitivity of glucose transport to insulin, did not improve with insulin therapy. The latter occurred despite an increase in insulin binding ($P < 0.01$). Finally, the improvement in maximal insulin-stimulated glucose transport correlated with the fall in fasting hyperglycemia ($r = 0.77$, $P < 0.01$). These findings demonstrate that several of the abnormalities of carbohydrate and lipid metabolism recently noted to be present in adipocytes from patients with NIDDM can be shown to significantly improve with insulin treatment.

INTRODUCTION

Results from several laboratories (1–7) have indicated that multiple defects exist in the action of insulin on adipocytes isolated from patients with noninsulin-dependent diabetes mellitus (NIDDM).[1] Thus, it has been shown that maximal insulin-stimulated glucose transport and sensitivity of glucose transport to insulin (1, 2), insulin-stimulated glucose oxidation (1, 3), both basal and insulin-stimulated glucose incorporation into triglycerides (1, 4), and insulin binding (5) are reduced and basal lipolysis is increased (6, 7) in adipocytes from such individuals.

Although these observations seem to document a series of steps in intracellular adipocyte metabolism that are abnormal in patients with NIDDM, it is not clear if these defects are the cause of the NIDDM, or secondary to the multiple biochemical derangements present in this syndrome. One way to differentiate between these alternatives would be to study various aspects of adipocyte metabolism before and after control of hyperglycemia in patients with NIDDM. In this regard, Arner et al. (8) recently reported that sulfonylurea treatment decreased basal lipolysis in adipose tissue isolated from patients with NIDDM to values seen in obese subjects with normal glucose tolerance, and Scarlett et al. (9) have reported that insulin-stimulated glu-

Received for publication 16 February 1983 and in revised form 20 July 1983.

[1] *Abbreviations used in this paper:* ED_{50}, concentration of insulin that resulted in a half-maximal transport rate; NIDDM, noninsulin-dependent diabetes mellitus.

cose transport improves after insulin treatment in six patients with NIDDM. However, both studies focus their attention on only one aspect of adipocyte metabolism. The current studies were undertaken in an effort to broaden the inquiry, and to measure several aspects of adipocyte metabolism in patients with NIDDM before and 4 wk after intensive control of hyperglycemia with multiple daily injections of insulin. The results to be presented indicate that maximum insulin-stimulated glucose transport and glucose incorporation into triglyceride and CO_2 were increased, and the rate of basal lipolysis reduced when isolated adipocytes from patients with NIDDM were studied before and after control of hyperglycemia with insulin. However, glucose incorporation into lactate and the sensitivity of glucose transport to insulin were not improved after insulin treatment; the latter occurred despite an increase in insulin binding.

METHODS

Subjects. 10 Southwestern American Indians with previously untreated NIDDM (clinical characteristics of individual subjects are shown in Table I) were studied while inpatients at the Phoenix Clinical Research Section. Except for diabetes and obesity, all subjects were in good health as judged by history and physical examination and standard laboratory determinations. Informed consent was obtained from all subjects.

Clinical procedures. Patients consumed a diet containing (as percentage of total calories) 45% carbohydrate, 30% fat, and 25% protein. Meals were served at 7:00 a.m., 12:00 p.m., 5:00 p.m., and 9:00 p.m. (distribution of 30, 30, 30, and 10% of total calorie intake, respectively). Patients were initially placed on a diet containing 30 kcal/kg and weighed daily; their calorie intake was modified to maintain body weight constant throughout the study. After at least 6 d on this diet, and after an overnight fast, each subject had subcutaneous abdominal adipose tissue removed from the lateral aspect of the hypogastrium, inferior to the umbilicus (McBurney's point). The skin was infiltrated with 2% xylocaine; care was taken not to infiltrate subcutaneous tissue. An elliptical incision ~4 cm in length was made and a wedge-shaped sample of subcutaneous fat underlying the incision (5–15 g) was dissected.

Insulin treatment was then initiated, with each patient receiving three subcutaneous injections of monocomponent insulin per day. The first injection was a mixture of Ultra-tard and Actrapid, and the other two injections were Actrapid only. The aim was to maintain fasting plasma glucose values near 100 mg/dl and 2-h postprandial glucose values to <140 mg/dl. The total dose of insulin administered varied between 99 and 222 U/d, with a mean of 198±20 U/d. Ultra-tard insulin was withdrawn after 1 mo, and patients were then given three daily injections of Actrapid. The fat biopsy was repeated on the contralateral side of the abdomen 4 d after removal of Ultra-tard and following an overnight fast. Fasting plasma glucose and insulin concentrations were determined the morning before each fat biopsy. Glucose was analyzed by the glucose oxidase method (10) by using a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, CA), and insulin concentration was analyzed by radioimmunoassay, using the dextran-coated charcoal separation technique (11).

Preparation of isolated adipocytes. Isolated adipocytes were prepared by the collagenase method modified for use in human adipocytes by Pedersen and Gliemann (12) with some modification. Briefly, adipose tissue was washed two times with 10 ml of Dulbecco's phosphate-buffered saline (Gibco Laboratories, Grand Island, NY) at room temperature, and all obvious connective tissue and blood clots were removed. The sample was cut into small pieces, and up to 6 g of tissue was added to 6 ml of collagenase solution (Hepes buffer containing 3.5% albumin, 1 mg/ml of collagenase, and 0.55 mM glucose) in 30 ml polyethylene Nalgene bottles. The bottles were shaken at 140 cycles/min at 37°C for 75 min. The cell suspension was passed through 450-μm nylon mesh. The cells were allowed to float for 3 min. The infranatant was removed and 20 ml of fresh buffer containing 5% albumin was added. This washing procedure was repeated three times. The cells were passed through nylon mesh a second time, and then concentrated to a 40% lipocrit for final dispersion to vials for incubations. Cells to be utilized

TABLE I
Description of Subjects

Subject	Sex	Age	Initial body mass index	Weight change	Fasting plasma				Known duration of diabetes
					Glucose		Insulin		
					Before	After	Before	After	
		yr	kg/m²	kg					yr
1	Female	38	40.5	+2.4	285	122	20	21	12
2	Male	19	40.2	+0.9	303	104	14	27	1
3	Female	37	52.0	+3.0	200	94	33	44	1
4	Female	24	47.3	−0.6	287	97	32	51	1
5	Male	42	34.5	+3.3	246	133	37	40	6
6	Female	37	53.7	+1.9	161	126	49	45	2
7	Female	28	43.9	−0.9	149	89	67	40	2
8	Male	42	26.0	+2.7	264	118	16	20	10
9	Female	32	49.0	+0.6	246	105	23	26	2
10	Male	33	41.0	+0.2	141	81	46	37	2

in glucose metabolism studies were washed and reconcentrated to 30% in Krebs-Ringer bicarbonate buffer (pH 7.4) equilibrated with 95% O_2/5% CO_2. The cells were never allowed to float without shaking for more than 5 min as this resulted in cohesion between the cells that causes them to break.

Determination of cell size. Isolated adipocytes were fixed with osmium tetroxide, washed, and diluted in saline as described by Cushman and Salans (13). Cells were sized by using a Coulter Electronic Cell Counter (model ZB; Coulter Electronics, Inc., Hialeah, FL) with a 400-μm aperture equipped with logarithmic range-expanded channelyzer. The volume of cells in the 100 channels was then calculated from a known volume in a reference channel. The average volume of cells in all channels were divided by the total number of cells sized. Cell volume was then converted to micrograms lipid per cell by using the correction factor of 0.91 μg lipid/nl cell volume. No clumping of adipocytes or partial digestion of the fat tissue was observed by viewing the fixed adipocytes under a microscope.

Measurement of insulin binding. Insulin binding was determined in quadruplicate by incubating a 300-μl suspension of isolated adipocytes (7%) in 5% albumin Hepes buffer in the presence of 0.5 mg/ml bacitracin, 50 pM [mono^{125}I-$TyrA_{14}$]insulin (0.6–0.9 μCi/pmol) plus 0, 50 pM, 150 pM, or 10 μM unlabeled insulin as described previously (14, 15). Incubations were carried out for 50 min at 37°C, with constant shaking at 160 times/min, and then, were terminated with cold saline; the cells were separated from the medium by centrifugation through oil (14, 15). The cells were collected in a disposable pipette tip and assayed for radioactivity in a auto-γ-counter (Packard Instrument Co., Inc., Downers Grove, IL). Insulin binding at 50, 100, and 200 pM insulin was corrected for nonspecific binding by subtracting values obtained in the presence of 10 μM insulin. Insulin binding was expressed as bound/free both per cell and per cell surface area.

Measurement of glucose transport. We have developed a new method for determination of cellular glucose transport based on the premise that glucose uptake provides a measurement of glucose transport when studies are carried out at very low glucose concentrations. We have chosen this method because it avoids the timing problems and complexities of the rapid pulse technique (16), utilizes <50% as many cells per sample as the 3-*O*-methylglucose method, makes it easier to do insulin dose-response experiments, and greatly lowers the isotope cost. A detailed validation of this method was reported (1), but a comparison between results obtained with this approach and the more conventional 3-*O*-methylglucose rapid pulse method (16) is seen in Table II. These results clearly demonstrate that the two methods provide comparable values for glucose transport, both basally and after insulin stimulation. To measure glucose transport with this technique, isolated adipocytes (2% lipocrit) were incubated in 500 μl 5% albumin buffer in the presence of 0, 25, 50, 100, 200, 800, and 8,000 pM insulin and trace (300 nM) amounts of 0.1 μCi [U-^{14}C]-D-glucose. The cell suspension was incubated at 37°C for 1 h with continuous shaking at 40 cycles/min. The incubation was terminated by centrifuging a 400-μl aliquot in a 550-μl microfuge tube containing 100 μl of silicone oil and the amount of radioactivity associated with the adipocytes (as well as the total radioactivity in the incubation medium) was determined by liquid scintillation counting. The glucose transport rate was expressed as the glucose clearance rate in femtoliters per cell · second: (clearance = volume in medium · cpm in cells/cpm in medium).

Calculation of insulin sensitivity. Since initial experiments indicated that an insulin concentration of 8,000 pM always resulted in a maximal stimulatory response, studies aimed at defining insulin sensitivity were carried out at insulin concentrations of 25, 50, 100, 200, 800, and 8,000 pM insulin. The concentration of insulin that resulted in a half-maximal transport rate (ED_{50}) was calculated for each individual subject from linear regression of transport rate vs. the log of the insulin concentrations at 25, 50, 100, 200, and 800 (if 800 pM insulin resulted in <80% of value) pM insulin. The correlation coefficient between these two variables was >0.95 in all experiments. A typical curve is seen in Fig. 1. The mean of these individual determinations of ED_{50} were defined for the entire group before and after treatment, and these values were used to evaluate the effect of insulin treatment on insulin sensitivity.

TABLE II

Comparison of the Rapid Pulse 3-O-Methylglucose Method and the 1-h Trace Glucose Method in Basal and Insulin-stimulated Adipocytes from Obese Normal Glycemic and Obese NIDDM Subjects

	3-O-Methylglucose		Glucose	
Subjects	Basal	8,000 pM insulin	Basal	8,000 pM insulin
Nondiabetic	57±17	152±36	43±17	134±32
Diabetic	25±7	55±12	20±6	42±16

° Values are given ±SEM; n = 3.

Calculation of glucose utilization. Total glucose utilization was determined by incubating cells with [U-^{14}C]-D-glucose in the presence of 5.5 mM glucose. Total glucose uptake was calculated by a summation of moles of glucose transported which were released as CO_2, which were retained in the cells, and which were released in the incubation medium as lactate. Nonlipid intracellular intermediates were calculated from the difference between glucose carbons that were retained in the cell and glucose incorporation into triglyceride.

Measurement of glucose oxidation and glucose incorporation into triglyceride. Isolated adipocytes (4%) were incubated in 1 ml Krebs-Ringer bicarbonate buffer (pH 7.4) equilibrated with 95% O_2 and 5% CO_2 containing 5% albumin, 0.5 μCi [U-^{14}C]-D-glucose and 5.5 mM glucose in the presence

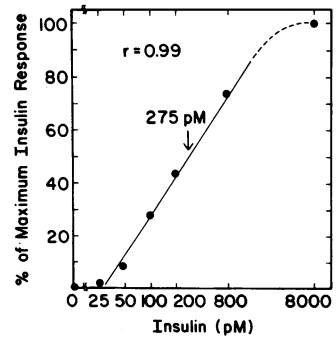

FIGURE 1 Glucose transport rate per cell as a function of insulin concentration (0, 25, 50, 100, 200, 800, and 8,000 pM) in a typical diabetic subject.

of 8 nM insulin at 37°C for 2 h with a continuous shaking at 40 cycles/min. The incubation was terminated by adding a 300 μl of 8 N sulfuric acid. $^{14}CO_2$ production was measured as described previously by Gliemann (17) and glucose incorporation into triglyceride was determined by the method described by Dole and Meinertz (18). Under these conditions, the total glucose utilization by the cells was linear for at least 3 h.

Measurement of glucose carbons retained in adipocytes and released from adipocytes as lactate. Isolated adipocytes (4%) were incubated in 500 μl Krebs-Ringer bicarbonate buffer (pH 7.4) equilibrated with 95% O_2 and 5% CO_2 containing 5% albumin, 0.25 μCi [U-^{14}C]-D-glucose, and 5.5 mM glucose in the presence of 8 nM insulin at 37°C for 2 h with a continuous shaking at 40 cycles/min. The incubation was terminated following the oil-flotation method as described previously (19). After separation by centrifugation, the packed cell layer was used for the measurement of the glucose retained in the cells, and the incubation medium below the oil layer was used for the measurement of lactate. Lactate was determined spectrophotometrically, following incubation of 200 μl of the medium with β-NAD and lactic dehydrogenase in glycine and hydrazine buffer (pH 9.2), using a pyruvate and lactate assay kit (20).

Measurement of basal lipolysis. Isolated adipocytes (4%) were incubated in 500 μl 5% albumin-Hepes buffer at 37°C for 2 h with continuous shaking at 40 cycles/min. The incubation was terminated by the oil flotation method as described previously (17), and the incubation medium under the oil was taken for determination of glycerol. Glycerol was determined by an enzymatic assay essentially as described previously by Wieland (21).

Statistics. All data are expressed as means±SEM. *r* values are Person product-moment correlations with significance probabilities. Significance of means was tested by using a paired *t* test.

Chemicals. Hepes buffer (pH 7.4) with albumin was prepared as described previously (14). Albumin (bovine, fraction V) was obtained from Armour Pharmaceuticals, Kankakee, IL; collagenase (type I) from Worthington Biochemical Corp., Freehold, NJ; porcine insulin from Eli Lilly & Co., Indianapolis, IN; L-glycine, ATP, β-NAD, glycerokinase, α-glycerophosphate dehydrogenase, glycerol, bacitracin, and osmium tetroxide were all from Sigma Chemical Co., St. Louis, MO; hydrazine hydrate from Fisher Scientific Co., Pittsburgh, PA; silicone oil from Union Carbide Corp., New York; 3-*O*-[U-^{14}C-*methyl*]-D-glucose (300–360 mCi/mmol), [l-^{3}H(N)]-L-glucose (100–200 mCi/mmol), and [U-^{14}C]-D-glucose (329 mCi/mmol) was from New England Nuclear, Boston, MA; [mono ^{125}I-*Tyr* A_{14}]insulin (Novo International Corp., Copenhagen, Denmark); all other chemicals were of analytical grade.

RESULTS

Mean (±SEM) fasting plasma glucose concentrations fell ($P < 0.001$) from 236±22 to 112±6 mg/dl after 1 mo of insulin treatment. There was no change in fasting plasma insulin concentrations after insulin therapy (Table I).

Fig. 2 shows glucose transport rate per cell of the 10 patients before and after 1 mo of insulin therapy. Basal glucose transport (Fig. 2 *A*) was 28.6±4.5 fl/cell·s before treatment and 35.3±5.0 fl/cell·s after treatment (NS). However, Fig. 2 *B* indicates that max-

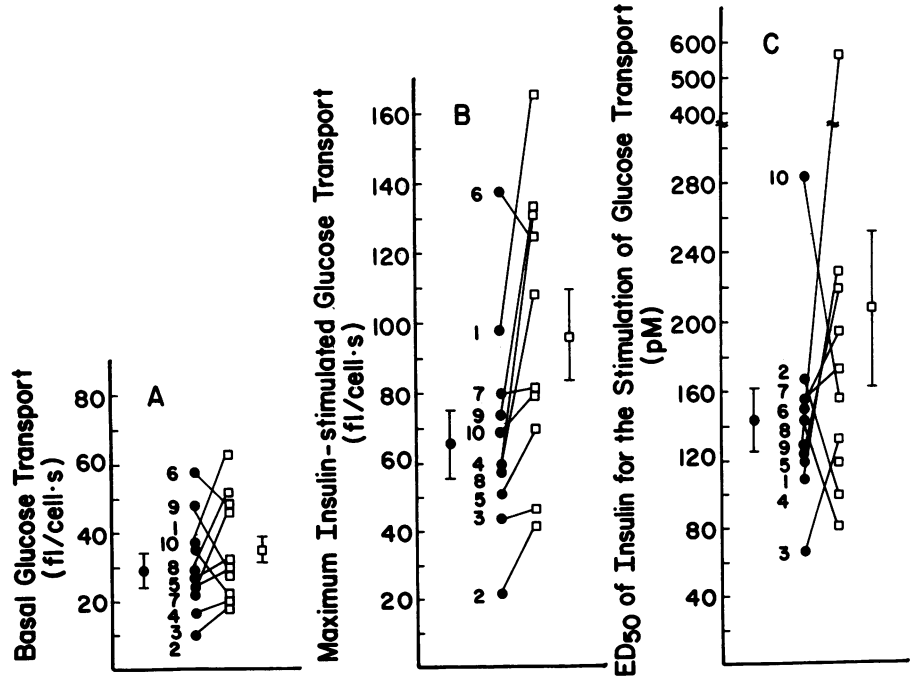

FIGURE 2 Basal (*A*) and maximum insulin-stimulated (*B*) glucose transport rates and ED_{50} of insulin for glucose transport (*C*), before (●) and after (□) insulin therapy. Numbers refer to patient number in Table I and means are ±SEM of 10 patients.

imum insulin-stimulated glucose transport significantly increased from 66.4±10.3 fl/cells·s before therapy to 97.0±13.2 fl/cell·s after therapy ($P < 0.02$). This resulted in a 61% ($P < 0.02$) increase in the increment between basal and maximum insulin-stimulated glucose transport as the result of therapy. However, the percent increase in maximal insulin-stimulated glucose transport over basal levels did not change as a result of therapy with insulin.

The effect of insulin treatment on sensitivity of the glucose transport system to insulin (ED_{50}) is seen in Fig. 2 *C*. Although there was a modest mean increase in ED_{50} following insulin treatment, this change was not statistically significant. Thus, insulin sensitivity was not modified by treatment.

Fig. 3 indicates that the relative improvement in maximum insulin-stimulated glucose transport after therapy was highly correlated with the relative decrease in fasting plasma glucose concentrations ($r = 0.77$, $P < 0.01$). Thus, the more effective insulin was in restoring euglycemia, the greater the degree of improvement in maximal insulin-stimulated glucose transport by adipocytes isolated from patients with NIDDM.

The effect of intensive insulin treatment on maximal insulin-stimulated glucose utilization is seen in Table III. These results indicate that insulin-stimulated conversion of glucose to CO_2 ($P < 0.05$) and triglyceride ($P < 0.01$) both increased by 50% after insulin administration. In contrast, no change was noted in conversion of glucose to lactic acid or other nonlipid metabolites. Thus, total maximal insulin-stimulated glucose utilization was not significantly increased by insulin treatment.

The results shown in Fig. 4 indicate that basal lipolysis was reduced in seven of nine subjects after insulin therapy, and the mean (±SEM) values of 290±70 attomol of glycerol release/cell·s (before therapy) and 110±20 attomol of glycerol release/cell (after therapy) were statistically significant ($P < 0.05$). However, there was no correlation between the percent improvement in basal lipolysis and improvement in fasting plasma glucose concentrations ($r = 0.27$).

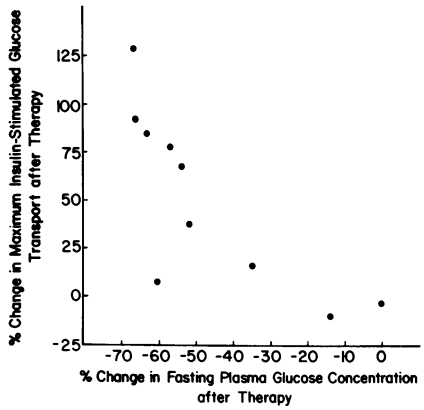

FIGURE 3 Percent change in maximum insulin-stimulated transport as a function of the percent change in fasting plasma glucose concentrations. Percent change is calculated as ([100* value after therapy]/[value before therapy]) − 100. r, −0.77; $P < 0.01$.

Insulin binding was studied at three insulin concentrations (50, 100, and 200 pM), and expressed at insulin bound over free both per cell and per surface area. The effect of insulin treatment on insulin binding to adipocytes incubated at 50 pM of insulin is seen in Fig. 5 and these results document a significant increase ($P < 0.02$) in binding after therapy. However, insulin binding was not significantly higher at 100 and 200 pM insulin either per cell or per surface area. The change in insulin binding seen after treatment must be viewed in the context of insulin action on glucose transport. Thus, the rate of glucose transport at a concentration of 50 pM of insulin averages ~15% of the maximal transport rate in these subjects. Furthermore, there is no correlation between the change in insulin binding observed at 50 pM of insulin and the change in sensitivity ($1/ED_{50}$) of the glucose transport system to insulin, either before or after insulin treatment. Consequently, the physiological significance of the change in binding is questionable.

DISCUSSION

The results of the present investigation demonstrate that adipocytes isolated from patients with NIDDM after 1 mo of insulin treatment show an increase in maximal insulin-stimulated glucose transport, conversion of glucose to CO_2 and triglyceride, maximum insulin binding, and a reduction in basal lipolysis. These data significantly extend the observations of Arner et al. (8) and Scarlett et al. (9), and provide strong support for the view that the abnormalities of carbohydrate and lipid metabolism present in adipocytes from patients with NIDDM (1–7) are at least partly a consequence of the diabetic state, and can be returned towards normal with effective insulin treatment. On the other hand, the design of the current study does not permit us to conclude that the intracellular abnormalities present in adipocytes from patients with NIDDM are entirely secondary to the presence of diabetes. For example, the decrease in sensitivity of the glucose transport system and in maximal insulin-stimulated glucose utilization found in adipocytes of patients with NIDDM did not improve significantly with insulin treatment. In addition, it must be emphasized that the improvement in maximal insulin-stimulated glucose transport and basal lipolysis noted in this study restored these values to levels of obese normal subjects (reference l;

TABLE III

5.5 mM Glucose Uptake and Conversion to Metabolic Products in Insulin-stimulated (8,000 pM) Adipocytes

	Glucose incorporation				
	Total	CO_2	Triglyceride	Lactic acid release	Nontriglycerides metabolites of glucose retained in the cell
	attomol/cell·s				
Before therapy	90.6±9.2	2.7±0.5	15.5±2.1	59.3±5.6	14.2±1.8
After therapy	113±17.1	4.1±0.8	24.6±6.9	67.7±12.6	16.7±1.8
Paired *t* test (p)	NS	<0.05	<0.01	NS	NS

Fig. 4 legend), but not to values seen in nonobese normal subjects studied in our laboratory (Foley et al., unpublished observation). Thus, it would be premature to conclude that all of the intracellular metabolic abnormalities present in adipocytes of patients with NIDDM are secondary phenomena.

In addition to improving various aspects of adipocyte metabolism, recent reports (22–25) indicate that insulin treatment can ameliorate the loss of normal in vivo insulin action that characterizes patients with NIDDM (26). Consequently, it is necessary to consider the possibility that the insulin-induced improvement in diabetic control and the enhancement of in vivo insulin action that occurs when patients with NIDDM are treated with insulin are related to the changes in glucose transport and/or insulin binding. In particular, a case can be made in this regard for the increase in glucose transport. Thus, a significant relationship between the magnitude of hyperglycemia and a decrease in maximal insulin-stimulated glucose transport in adipocytes isolated from untreated patients with NIDDM has been previously described (1, 2), and the present results demonstrate that the improvement in diabetic control with insulin was significantly related to an increase in maximal insulin-stimulated glucose transport by isolated adipocytes. A similar relationship has also been noted by Scarlett et al. (9). If parallel changes in maximal insulin-stimulated glucose utilization also exist in the

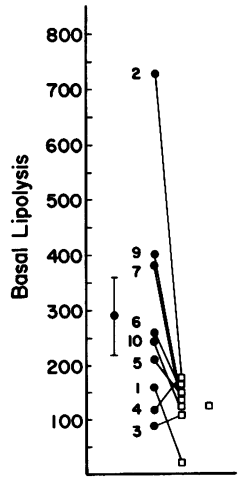

FIGURE 4 Basal lipolysis rates (attomol/cell·s glycerol release) before (●) and after (□) insulin therapy. Numbers refer to patient number in Table I and means are ±SEM of 10 patients. For comparison, the basal lipolysis rate of the 10 normal glycemic obese subjects whose glucose transport and metabolism are shown in reference 1 were 130±20 attomol/cell·s.

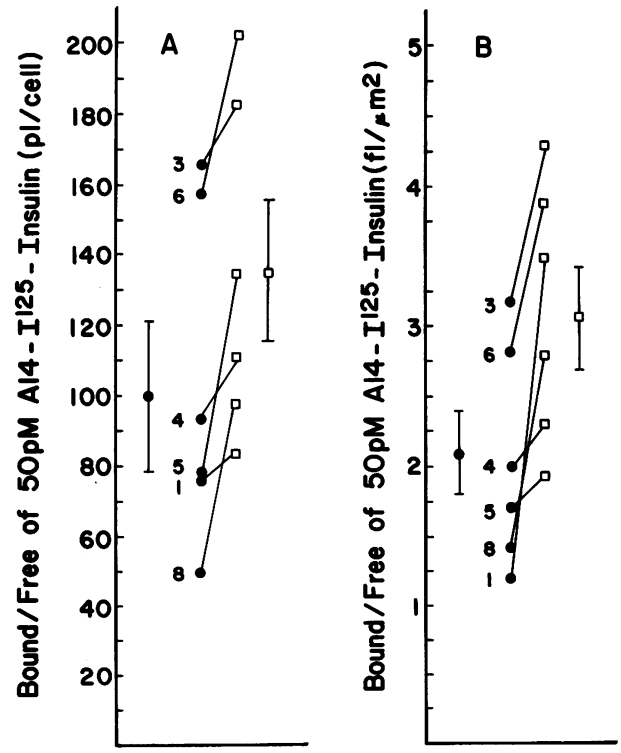

FIGURE 5 50 pM insulin binding before (●) and after (□) insulin therapy. Numbers refer to patient number in Table I and means are ±SEM of 10 patients.

muscle of untreated patients with NIDDM, it is obvious that these two tissue defects could contribute to the hyperglycemia and insulin resistance that exists before treatment, as well as the improvement in both of these functions seen after 1 mo of insulin administration.

It appears to us that a less persuasive case can be made for the part played by insulin receptors in control of the in vivo defects in carbohydrate metabolism of patients with NIDDM. In the first place, the results of insulin treatment on insulin binding by adipocytes from patients with NIDDM is not totally consistent. Thus, we observed that insulin treatment also led to a significant increase in the ability of isolated adipocytes from patients with NIDDM to bind trace insulin. Somewhat similar results were recently reported by Scarlett et al. (22) in six patients with NIDDM who were treated with insulin for 14 d. However, the improvement in insulin binding noted by these researchers did not reach statistical significance. The reason for this discrepancy is not clear. The severity of the diabetes in patients studied by Scarlett and associates appeared comparable to that of our subjects, as was the improvement after insulin treatment. Perhaps, the increase in insulin binding that they noted would have become statistically significant if they had continued the treatment program longer. In any event, both studies demonstrate that treatment with large doses of exogenous insulin does not lead to a loss of insulin binding, as might have been anticipated to occur due to down-regulation of the insulin receptor (27). For example, the lowest dose of insulin that any patient in our study received was 99 U/d, and the mean dose was 198 U/d. The insulin used was monocomponent and insulin antibodies did not develop during the course of the study. Thus, ambient insulin levels must have been substantially greater than those seen in normal subjects. On the other hand, down-regulation of the number of insulin receptors may have taken place, but have been obscured by an increase in insulin receptor number associated with the improvement in metabolic control. Irrespective of the mechanism involved, both studies are consistent in pointing out that the need to use large amounts of insulin to treat obese patients with NIDDM will not accentuate the reduction in insulin binding described in patients with NIDDM (28) and, if anything, may lead to an increase in insulin binding.

However, even if insulin treatment of patients with NIDDM leads to an increase in insulin binding, it does not follow that this change would have a substantial impact on insulin action. Thus, recent models of insulin action (27) would predict that the sensitivity to insulin of the glucose transport system should increase as the result of the increase in the number of insulin receptors. However, we could not document this event, and actually observed a decreased sensitivity (increased ED_{50}) to insulin-stimulated glucose transport in five of the six patients in which insulin binding was measured. (Presumably, the decrease in insulin sensitivity associated with insulin therapy in these individuals was due to a postbinding defect in the coupling of receptor to effector.) In addition, no correlation was observed between insulin binding and plasma glucose levels before and/or after insulin treatment, and the improvement in diabetic control was not correlated with an increase in insulin binding. For all of these reasons, we don't think it likely that either the improvement in diabetic control or the insulin resistance can be attributed to the observed increase in insulin binding.

Although we believe that the data presented provide new insights into the impact of insulin therapy on tissue defects in patients with NIDDM, it is apparent that there is a good deal more to be learned about the effects of successful diabetic control with insulin on both in vivo and in vitro aspects of insulin action. For example, the fact that the improvement in glucose control correlated with the increase in maximal insulin-stimulated glucose transport does not mean that the two events are causally related. Furthermore, we cannot discern from these studies the mechanism responsible for the observed changes in maximal insulin-stimulated glucose transport associated with insulin treatment. The fact that maximal transport increased, while the percent response stayed the same, argues for an increase in the capacity of the transport system. With this foundation, it is not necessary to postulate any changes in insulin action on glucose transport by adipocytes. On the other hand, although the percent stimulation did not change, there was an increase in the incremental response of glucose transport to insulin following therapy, and Hissen et al. (29) have recently suggested that this type of change can be due to the acute effect of insulin on recruiting glucose transport units. Resolution of this point awaits further understanding of insulin's action on glucose transport. An unexplained observation is that the reduction in maximal insulin-stimulated glucose conversion to lactate and nonlipid intracellular intermediates was not restored as a result of insulin therapy. However, insulin treatment did lead to an increase in the conversion of glucose to CO_2 and triglycerides in insulin-treated patients. We have no explanation for this discrepancy, and our lack of understanding emphasizes the relative primitive nature of our knowledge of the changes in intracellular glucose and lipid metabolism that occur in patients with NIDDM.

Finally, three qualifications must be clearly stated. In the first place, it must be understood that our results do not differentiate between a specific effect of insulin on adipose tissue metabolism in patients with NIDDM, as distinguished from the impact of achieving excellent control of hyperglycemia. Secondly, Pedersen and

Hjollund (30) have recently compared insulin binding and in vitro insulin action of adipocytes isolated from normal subjects and patients with insulin-dependent diabetes mellitus. In contrast to the present study of NIDDM subjects, they observed decreased insulin binding, which correlated with decreased sensitivity of the glucose transport system and of lipolysis to insulin. Without a nontreated insulin-dependent diabetes mellitus group, it is difficult to determine whether these changes are due to diabetes and/or the insulin treatment. In any case, the differences between their data and ours raises the possibility that changes in in vitro adipocyte function may not be the same in subjects with different types of diabetes mellitus.

Thirdly, it should be remembered that the present study was carried out on a relatively homogenous group of Southwestern American Indians. Thus, it is always possible that the observed changes in adipocyte function, both before and after insulin therapy, are unique to this population. However, the fact that a similar improvement in maximum insulin-stimulated glucose transport after insulin therapy has also been found in a population of genetically heterogeneous Caucasian patients with NIDDM (9) makes this possibility less likely. Consequently, we would suggest that the changes in insulin action and binding that we have described are likely to be characteristic of the obese patient with NIDDM, and further in vitro studies of adipocyte metabolism in patients with NIDDM should provide important information as to the pathogenesis of this syndrome.

ACKNOWLEDGMENTS

We wish to thank the volunteers who made this study possible, Mr. Thomas P. Hueckstead and Mr. Dave Brady for their excellent technical assistance, and Miss Phyllis J. Loco for secretarial assistance.

REFERENCES

1. Kashiwagi, A., M. A. Verso, J. Andrews, B. Vasquez, G. Reaven, and J. E. Foley. 1983. In vitro insulin resistance of human adipocytes isolated from subjects with non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* 72:1246–1254.
2. Ciaraldi, T. P., O. G. Kolterman, J. A. Scarlett, M. Kao, and J. M. Olefsky. 1982. Role of glucose transport in the post-receptor defect of non-insulin-dependent diabetes mellitus. *Diabetes.* 31:1016–1022.
3. Bolinder, J., J. Ostman, and P. Arner. 1982. Postreceptor defects causing insulin resistance in normoinsulinemic noninsulin-dependent diabetes mellitus. *Diabetes.* 31:911–916.
4. Lonnrath, P., M. DiGiralamo, M. Krotkiewski, and U. Smith. 1983. Insulin binding and responsiveness in fat cells from patients with reduced glucose tolerance and type II diabetes. *Diabetes.* 32:748–754.
5. Olefsky, J. M., O. G. Kolterman, and J. A. Scarlett. 1982. Insulin action and resistance in obesity and noninsulin-dependent type II diabetes mellitus. *Am. J. Physiol.* 243:E1–E30.
6. Carlson, L. A., and J. Ostmon. 1963. In vitro studies on the glucose uptake and fatty acid metabolism of human adipose tissue in diabetes mellitus. *Acta Med. Scand.* 174:215–218.
7. Bjorntorp, P., and B. Hood. 1966. Studies on adipose tissue from obese patients with and without diabetes mellitus. Release of glycerol and free fatty acids. *Acta Med. Scand.* 179:221–224.
8. Arner, P., P. Engfeldt, and J. Ostmon. 1980. Blood glucose control and lipolysis in diabetes mellitus. *Acta Med. Scand.* 208:297–299.
9. Scarlett, J. A., O. G. Kolterman, T. P. Ciarldi, M. Koa, and J. M. Olefsky. 1983. Insulin treatment reverses the postreceptor defect in adipocyte 3-*O*-methylglucose transport in type II diabetes mellitus. *J. Clin. Endocrinol. Metab.* 56:1195–1201.
10. Kadish, A. H., R. L. Little, and J. C. Sternberg. 1968. A new and rapid method for the determination of glucose by measurement of rate of oxygen consumption. *Clin. Chem.* 14:116–131.
11. Herbert, V., K. Lau, C. W. Gottlich, and S. J. Bleichem. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* 25:1375–1384.
12. Pedersen, O., and J. Gliemann. 1981. Hexose transport in human adipocytes: factors influencing the response to insulin and kinetics of methylglucose and glucose transport. *Diabetologia.* 20:630–635.
13. Cushman, S. W., and L. B. Salans. 1978. Determination of adipose cell size and number in suspensions of isolated rat and human adipose cells. *J. Lipid Res.* 19:269–273.
14. Gliemann, J., and O. Sonne. 1978. Binding and receptor-mediated degradation of insulin in adipocytes. *J. Biol. Chem.* 253:7857–7863.
15. Pedersen, O., E. Hjollund, H. Beck-Nielsen, H. O. Lindskov, O. Sonne, and J. Gliemann. 1981. Insulin receptor binding and receptor-mediated insulin degradation in human adipocytes. *Diabetologia.* 20:636–641.
16. Kashiwagi, A., and J. E. Foley. 1982. Opposite effects of beta adrenergic agonist and phosphodiesterase inhibitor on glucose transport in isolated human adipocytes: isoproterenol increases V_{max} and IBMX increases K_t. *Biochem. Biophys. Res. Commun.* 107:1151–1157.
17. Gliemann, J. 1967. Assay of insulin-like activity by the isolated fat cell method. 1. Factors influencing the response to crystalline insulin. *Diabetologia.* 3:382–388.
18. Dole, V. P., and H. Meinertz. 1960. Microdetermination of long-chain fatty acids in plasma and tissues. *J. Biol. Chem.* 235:2595–2599.
19. Foley, J. E., A. L. Lausen, O. Sonne, and J. Gliemann. 1980. Insulin binding and hexose transport in rat adipocytes: relation to cell size. *Diabetologia.* 19:234–241.
20. Henry, R. J. 1968. Carbohydrate metabolites. *In* Clinical Chemistry Principles and Technics. Norman Weissman, editor. Harper & Row Publishers, Inc., New York. 664–666.
21. Weiland, O. 1974. Glycerol (UV-method). Method of Enzymatic Analysis. Bergmeyer, editor. Academic Press Inc., New York. 1404–1408.
22. Scarlett, J. A., R. S. Gray, J. Graffin, J. M. Olefsky, and O. G. Kolterman. 1982. Insulin treatment reverses the insulin resistance of type II diabetes mellitus. *Diabetes Care.* 5:353–363.
23. Andrews, J., I. Klimes, B. Vasquez, and M. Nagulesparan.

1982. Can one month strict glycemia control in non-insulin-dependent diabetes return insulin action to normal diabetes. American Diabetes Association, 42nd Annual Meeting, San Francisco. 59A.

24. Ginsberg, H., and E. J. Rayfield. 1981. Effect of insulin therapy on insulin resistance in type II diabetic subjects: evidence for heterogeneity. *Diabetes.* 30:739–945.
25. Greenfield, M., C. Landinois, L. Doberne, and H. Vreman. 1982. Metabolic effects of intensive insulin therapy in patients with non-insulin-dependent diabetes (NIDDM). American Diabetes Association, 42nd Annual Meeting, San Francisco, 58A.
26. Reaven, G. M. 1980. Insulin-dependent diabetes mellitus: metabolic characteristics. *Metab. Clin. Exp.* 29:445–454.
27. Roth, J., C. R. Kohn, M. A. Lesniok, P. Gorden, P. DeMeyts, K. Meggesi, D. Weville, J. R. Gavin, S. H. Soll, P. Freychet, I. E. Goldfine, R. S. Bar, and J. A. Archer. 1976. Receptors for insulin NSILAs and growth hormone: applications to disease states in man. *Recent Prog. Horm. Res.* 31:95–126.
28. Olefsky, J. M., and G. M. Reaven. 1977. Insulin binding in diabetes: relationships with plasma insulin levels and insulin sensitivity. *Diabetes.* 26:680–688.
29. Hissen, P. J., J. E. Foley, L. J. Wardzalla, E. Kornieli, I. A. Simpson, L. B. Salans, and S. W. Cushman. 1982. Mechanism of insulin-resistant glucose transport activity in the enlarged adipose cell of the aged obese rat: relative depletion of intracellular glucose transport systems. *J. Clin. Invest.* 70:780–790.
30. Pedersen, O., and E. Hjollund. 1982. Insulin receptor binding to fat and blood cells and insulin action in fat cells from insulin-dependent diabetics. *Diabetes.* 31:706–715.